Kidney International, Vol. 62 (2002), pp. 2230–2237

Antibody to oxidized low-density lipoprotein and cardiovascular mortality in end-stage renal disease

Tetsuo Shoji, Mariko Fukumoto, Eiji Kimoto, Kayo Shinohara, Masanori Emoto, Hideki Tahara, Hidenori Koyama, Eiji Ishimura, Tatsuya Nakatani, Takami Miki, Yoshihiro Tsujimoto, Tsutomu Tabata, and Yoshiki Nishizawa

Department of Metabolism, Endocrinology and Molecular Medicine, Department of Nephrology, Osaka City University Graduate School of Medicine, Department of Urology, and Department of Geriatrics and Neurology, Osaka City University Medical School, Osaka; and Division of Internal Medicine, Inoue Hospital, Suita, Japan

Antibody to oxidized low-density lipoprotein and cardiovascular mortality in end-stage renal disease.

Background. Immune response to oxidized low-density lipoprotein (oxLDL) may modulate the process of atherogenesis and cardiovascular disease.

Methods. We performed a prospective, observational cohort study in 249 patients with end-stage renal disease (ESRD) to examine whether the serum titer of anti-oxLDL antibody can predict cardiovascular mortality.

Results. The median anti-oxLDL antibody titer was 320 mU/mL at baseline. During the follow-up (63 ± 23 months), 72 deaths including 34 cardiovascular deaths occurred. When the subjects were divided into two groups by the median titer, the high titer group showed a lower risk for cardiovascular mortality ($P = 0.040$ by Kaplan-Meier analysis and log-rank test). Multivariate Cox proportional hazards model indicated that the lower risk of cardiovascular death in the high titer group remained significant (hazard ratio of 0.46, 95% CI 0.23–0.95, $P = 0.037$) and independent of age, presence of vascular complications, presence of diabetes mellitus, and elevated C-reactive protein. In contrast, anti-oxLDL antibody titer was not associated with non-cardiovascular mortality.

Conclusions. These results demonstrate, to our knowledge for the first time, that serum anti-oxLDL antibody titer is an independent predictor of cardiovascular mortality in a cohort of patients with ESRD.

Since oxidized low-density lipoprotein (oxLDL) plays a central role in the initiation and progression of atherosclerosis [1], immune response to oxLDL may modulate atherogenesis [2] and cardiovascular mortality. In atheromatous plaques, there are immune competent cells including macrophages, activated T [3], and B lymphocytes expressing immunoglobulin mRNA [4], suggesting local production of antibodies. OxLDL is detected in atheromatous lesions [5–7], and it can activate lymphocyte clones [8]. Immunoglobulins extracted from plaques recognize oxLDL [9], and antibody against oxLDL is present in serum [6, 10–19]. To date it has not been established whether immune response to oxLDL is atherogenic or anti-atherogenic.

Atherosclerosis is advanced [20–23] and the cardiovascular mortality rate is substantially elevated among patients with end-stage renal disease (ESRD) [24, 25]. Some studies showed that ESRD patients had a higher titer of anti-oxLDL antibody than healthy subjects [26, 27]. Elevated anti-oxLDL antibody also was reported in patients with coronary artery disease [11], severe carotid atherosclerosis [10], and peripheral artery disease [12]. Although some studies failed to confirm such elevation in anti-oxLDL antibody in patients with acute myocardial infarction [16], cerebral infarction [15], diabetic macroangiopathy [14], and familial hypercholesterolemia [17], most of these observations suggest that titer of anti-oxLDL antibody is elevated in those with advanced atherosclerosis.

In contrast to the comparison between groups with and without advanced atherosclerosis, a few studies focused on the variation of anti-oxLDL antibody titer within healthy subjects. Recently, we found an inverse association between serum anti-oxLDL antibody and circulating oxLDL levels in healthy human subjects [18]. We also showed an independent and inverse association between anti-oxLDL antibody titer and intima-media thickness of carotid artery in a healthy population [19]. These results indicate that a high titer of anti-oxLDL antibody may be protective against atherosclerosis among the healthy subjects.

The reasons for the discrepancy between studies are unknown. However, all these studies are cross-sectional

Key words: hemodialysis, atherosclerosis, oxidized LDL, atherosclerosis, immune response, uremia, anti-oxLDL.

Received for publication April 15, 2002
and in revised form June 13, 2002
Accepted for publication July 31, 2002

Table 1. Characteristics of the cohort

	Total	Low titer	High titer
Number of subjects	249	125	124
Age *years*	55.4 ± 10.4	54.6 ± 9.9	56.2 ± 10.9
Duration of HD *years*	5.4 (0.3–21.9)	5.9 (0.3–21.9)	4.8 (0.5–20.3)
Body mass index kg/m^2	21.5 ± 2.7	21.2 ± 2.6	21.7 ± 2.8
Systolic BP *mm Hg*	153 ± 27	154 ± 25	151 ± 30
Diastolic BP *mm Hg*	85 ± 14	84 ± 15	86 ± 14
Total cholesterol *mmol/L*	4.37 ± 1.02	4.43 ± 0.96	4.26 ± 1.09
Triglycerides *mmol/L*	1.31 (0.85–11.9)	1.30 (0.42–4.27)	1.31 (0.37–5.19)
HDL cholesterol *mmol/L*	1.01 ± 0.29	1.05 ± 0.30	0.97 ± 0.27[a]
Non-HDL cholesterol *mmol/L*	3.34 ± 1.00	3.39 ± 0.98	3.29 ± 1.02
LDL cholesterol *mmol/L*	2.66 ± 0.85	2.74 ± 0.78	2.62 ± 0.88
Fasting glucose *mmol/L*	4.85 ± 1.97	4.63 ± 1.56	5.06 ± 2.29
Serum creatinine *μmol/L*	1021 ± 174	1017 ± 188	1024 ± 160
Serum albumin *g/L*	37.9 ± 2.9	37.5 ± 2.9	38.2 ± 2.9[b]
C-reactive protein *mg/L*	4.0 (1.0–53)	3.5 (1.0–43)	4.0 (1.0–53)
Hematocrit *%*	27.4 ± 4.0	27.2 ± 4.3	27.6 ± 3.8
Male *%*	40	38	43
Smoker *%*	23	25	20
Presence of diabetes *%*	18	15	20
Presence of dyslipidemia *%*	76	71	82[c]
Presence of hypertension *%*	83	87	77[c]
Presence of vascular complications *%*	18	18	17
Medications for dyslipidemia *%*	2.0	3.2	0.8
Medications for hypertension *%*	60	64	57

Data are summarized as mean ± SD, median (range), or percentage. The "low titer" and "high titer" groups were defined based on the median (320 mU/mL) of serum anti-oxLDL antibody titer. Definitions of diabetes mellitus, dyslipidemia and hypertension are indicated in the text. Abbreviations are: BP, blood pressure; HDL, high-density lipoprotein; Non-HDL, non-high-density lipoprotein; LDL, low-density lipoprotein.

[a] $P < 0.05$ and [b] $P = 0.064$ vs. low titer groups by ANOVA

[c] $P = 0.059$–0.070 vs. low titer group by chi-square test

and have limitations in evaluating the causality between anti-oxLDL antibody titer and atherosclerotic diseases. To date, no prospective study is available in the literature that examines whether anti-oxLDL antibody is associated with increased or decreased death from cardiovascular disease. In the present study, we performed a cohort study in 249 patients with ESRD to prospectively evaluate the possible association between anti-oxLDL antibody and cardiovascular mortality.

METHODS

Study design and subjects

This was a prospective, observational cohort study. The cohort consisted of 249 out of 265 ESRD patients in the MAP ESRD study (Metabolic changes, Arteriosclerosis and Prognosis in ESRD) that was described recently [23]. Sixteen subjects were excluded because anti-oxLDL antibody data were not available. They had been treated by regular hemodialysis for more than three months at Inoue Hospital, Suita, Japan. The subjects were recruited from those who were dialyzed during the morning sessions, so that blood tests were done after an overnight fast. Patients were excluded when they had severe illness or apparent acute inflammatory symptoms. The patients corresponded to 85% of those who received hemodialysis in morning sessions, or 50% of the total hemodialysis patients in this hospital. The subjects were registered between June 1992 and June 1995, and the age at entry was 55.4 ± 10.4 years (mean ± SD). Forty-four patients (18%) had type 2 diabetes mellitus, and their mean duration of diabetes was 21 ± 6 years at inclusion. The diagnosis of diabetes was made according to the World Health Organization criteria [28]. Exclusion criteria included those with type 1 diabetes who were positive for GAD antibody, who had a past history of ketoacidosis, or who were dependent on insulin therapy for survival. Gestational diabetes and diabetes associated with specific syndromes were carefully ruled out by their history. The patients received three to five hours of hemodialysis, three times a week, using bicarbonate dialysate. They gave informed consent to participate, and this study was approved by the institutional ethical committee (Inoue Hospital Approval No. 106). Table 1 summarizes baseline characteristics of the subjects.

Definition of dyslipidemia

The presence of dyslipidemia was diagnosed when a subject had one or more of the following criteria [29]; (*1*) low-density lipoprotein cholesterol of 100 mg/dL (2.58 mmol/L) or higher, (*2*) non-high-density lipoprotein (non-HDL) cholesterol of 130 mg/dL (3.36 mmol/L) or higher, (*3*) plasma triglycerides of 150 mg/dL (1.69 mmol/L) or higher, (*4*) HDL cholesterol of 40 mg/dL (1.03 mmol/L) or lower, and (*5*) use of one or more lipid-lowering drugs. According to the criteria, a total of 189

subjects, including five subjects taking statins, were diagnosed to have dyslipidemia at baseline.

Definition of hypertension

Blood pressure was measured with a standard mercury sphygmomanometer and cuffs adapted to arm circumference, after the subject had rested in a supine position for at least five minutes. The systolic and diastolic blood pressure levels were taken as the points of appearance and disappearance of Korotkoff sounds, respectively. The average of three measurements was used for analysis. The presence of hypertension was diagnosed when a subject had one or more of the following criteria: (*1*) systolic blood pressure of 140 mm Hg or higher, (*2*) diastolic blood pressure of 90 mm Hg or higher, and (*3*) use of one or more anti-hypertensive drugs [30]. According to the criteria, 207 subjects were diagnosed to have hypertension at baseline.

Definition of elevated C-reactive protein

The median of C-reactive protein level was 4.0 mg/L, which was also the upper limit of the normal range of the assay. C-reactive protein levels higher than 4 mg/L were defined as elevated in this study, and 125 patients had an elevated C-reactive protein concentration at baseline.

Pre-existence of vascular complications a baseline

The presence of vascular complications was evaluated by clinical information regarding coronary, cerebral, and peripheral artery diseases and aortic aneurysm. Coronary artery disease was diagnosed when a subject had one of more of the following criteria: (*1*) past history of percutaneous coronary intervention or coronary artery bypass grafting, (*2*) presence of significant stenosis by coronary angiography, (*3*) presence of ST-T abnormalities on electrocardiogram associating typical symptoms attributable to angina pectoris, and (*4*) use of one or more medications for coronary ischemia. Thirteen patients were diagnosed to have coronary artery disease. Cerebrovascular disease was diagnosed by past history that had been confirmed by positive findings of infarction or bleeding by x-ray computed tomography or magnetic resonance imaging. Seventeen patients had such past history. Peripheral artery disease was diagnosed in four patients with intermittent claudication and/or leg pain at rest when significant arterial stenosis was confirmed by angiography. Diagnosis of aortic aneurysm was made by x-ray computed tomography in one patient. At baseline, 44 patients had one or more of the above vascular complications.

Measurement of anti-oxLDL antibody

Anti-oxidized LDL antibody was measured by ELISA using a commercially available kit (OLAB, Biomedica, Vienna, Austria) as previously described [18, 19]. Test sera were prediluted with buffer, and incubated at 37°C for 90 minutes in 96-well microtiter plates precoated with copper-oxidized LDL. After washing, the wells were incubated with anti-human IgG antibody conjugated with a specific peroxidase at room temperature for 30 minutes. The wells were washed, added with tetramethylbentidine and incubated at room temperature for 15 minutes in the dark. Color development was stopped by adding sulfuric acid. The absorbance at 450 nm was read by a microplate reader. Antibody (Ab) titer was calculated by constructing a standard curve using the standards included in the kit; the unit for oxLDL Ab was defined by the manufacturer. Intra-assay and interassay reproducibility (CV) of the assay was $<5\%$ and $<10\%$, respectively [18]. The presence of oxidized LDL in the test serum did not interfere significantly with the assay as previously described [18].

Other measurements

Blood was drawn in the morning after an overnight fast of at least 12 hours before starting a dialysis session. Whole blood was used for hematocrit, ethylenediaminetetraacetic acid (EDTA)-plasma for lipids, and serum for other biochemical assays. Total cholesterol was measured enzymatically using a commercially available kit (Wako Pure Chemicals, Osaka, Japan). HDL cholesterol was measured after precipitating apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride. Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol. Other measurements were performed using routine methods.

Outcome data collection

The cohort was followed up to December 1998. During the follow-up, 47 patients moved away from Inoue Hospital: the outcome data of 34 out of the 47 patients could be obtained, while the remaining 13 patients including one who underwent renal transplantation were censored. At the end of the follow-up, 162 patients were confirmed to be alive on hemodialysis and 74 to be dead. The mean follow-up period was 63 ± 23 months.

Date and cause of death were obtained by reviewing the hospital record forms. For patients transferred to other dialysis units, we reviewed the questionnaire forms filled by the attending physicians at the units. The 74 deaths during the follow-up included 34 fatal cardiovascular events: 8 deaths attributable to coronary heart disease, 5 to cerebrovascular disease, 12 to congestive heart failure, and 9 to sudden death. Sudden death was defined as a witnessed death that occurred within one hour after the onset of acute symptoms, with no evidence of accident or violence. The 40 fatal non-cardiovascular causes were: infectious disease ($N = 17$), hepatic failure ($N = 6$), cancer ($N = 5$), and bleeding including disseminated

intravascular coagulation ($N = 4$), cachexia ($N = 3$), respiratory failure ($N = 3$), acute pancreatitis ($N = 1$) and suicide ($N = 1$).

Statistical analysis

Data were summarized as mean ± SD for continuous variables. The difference between mean values was assessed by analysis of variance (ANOVA). Data with a skewed distribution, such as serum anti-oxLDL antibody titer, were summarized as median (range), and the comparison between groups was performed using the nonparametric Mann-Whitney U test. Correlation between two parameters was tested by Spearman's rank correlation method. Survival curves were estimated by the Kaplan-Meier method followed by log-rank test. Prognostic variables for survival were examined using univariate and multivariate Cox proportional hazards regression models. Covariates that were significant in univariate analysis were forced into the multivariate Cox model. P values less than 0.05 were considered significant. All these analyses were performed using statistical software (StatView 5, SAS Institute Inc., Cary, NC, USA) for Windows personal computers.

RESULTS

Distribution of serum anti-oxLDL antibody titer

Serum anti-oxLDL antibody titer at baseline showed a skewed distribution with a median of 320 mU/mL (Fig. 1). This value in the hemodialysis population was significantly higher than that of 446 healthy subjects with similar age in our previous study (median 254 mU/mL, $P = 0.0002$ by Mann-Whitney U test) [19].

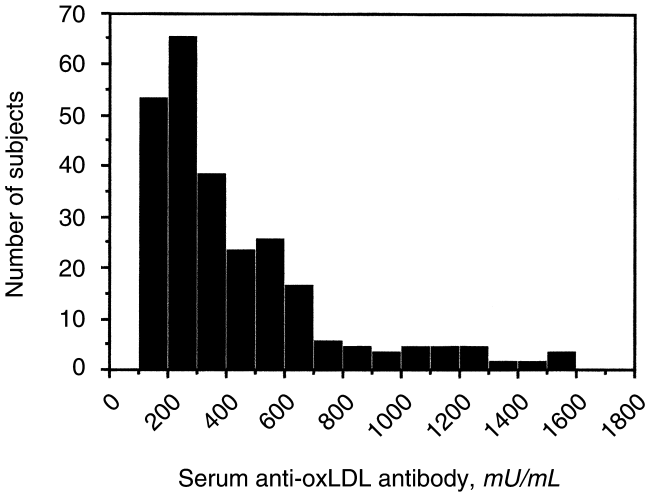

Fig. 1. Serum anti-oxidized low-density lipoprotein (anti-oxLDL) antibody titer in the cohort of patients with end-stage renal disease (ESRD). Serum anti-oxLDL antibody at baseline showed a skewed distribution with a median of 320 mU/mL (range 103 to 1558 mU/mL; $N = 249$).

Kaplan-Meier analysis of relationship between anti-oxLDL antibody and mortality

The subjects were divided into two groups according to serum anti-oxLDL antibody titer, and prognosis was compared by Kaplan-Meier analysis (Fig. 2). The high titer group had a significantly lower risk of cardiovascular death than the low titer group ($P = 0.040$). In contrast, such a trend was not found in non-cardiovascular mortality between the two groups ($P = 0.518$).

Predictors of cardiovascular mortality in univariate and multivariate Cox models

First, univariate Cox proportional hazards models were used to evaluate the association of cardiovascular mortality with anti-oxLDL antibody and 12 other variables (Table 2). Significant univariate predictors of cardiovascular mortality were age, elevated C-reactive protein, presence of diabetes mellitus, presence of vascular complications, and the titer of anti-oxLDL antibody. A higher anti-oxLDL antibody titer was significantly associated with a lower risk of cardiovascular death. Duration of hemodialysis treatment and gender tended to predict cardiovascular mortality at a borderline significance. Other variables including medications for hypertension or dyslipidemia did not show significant relationship with cardiovascular mortality. Also, cardiovascular mortality did not show a significant association with HDL cholesterol, non-HDL cholesterol, LDL cholesterol, triglycerides, systolic blood pressure, or diastolic blood pressure (data not shown).

Then, the five significant univariate predictors of cardiovascular mortality were forced into the multivariate Cox regression model to identify independent predictors (Table 3). The results indicated that the presence of vascular complications, age, and titer of anti-oxLDL antibody were independent predictors of cardiovascular mortality. Again, a higher titer of anti-oxLDL antibody was a predictor of a lower risk of death from cardiovascular causes with a hazards ratio of 0.46 (95% confidence interval, 0.23-0.95, $P = 0.037$).

Predictors of non-cardiovascular mortality in univariate and multivariate Cox models

Similar analyses were performed to examine the association of non-cardiovascular mortality with anti-oxLDL antibody and other variables (Table 2). Significant univariate predictors of non-cardiovascular death were age, serum albumin, C-reactive protein, and presence of diabetes mellitus, whereas other factors including anti-oxLDL antibody were not significantly associated with non-cardiovascular mortality. These four covariates remained significant in the multivariate Cox model (Table 4).

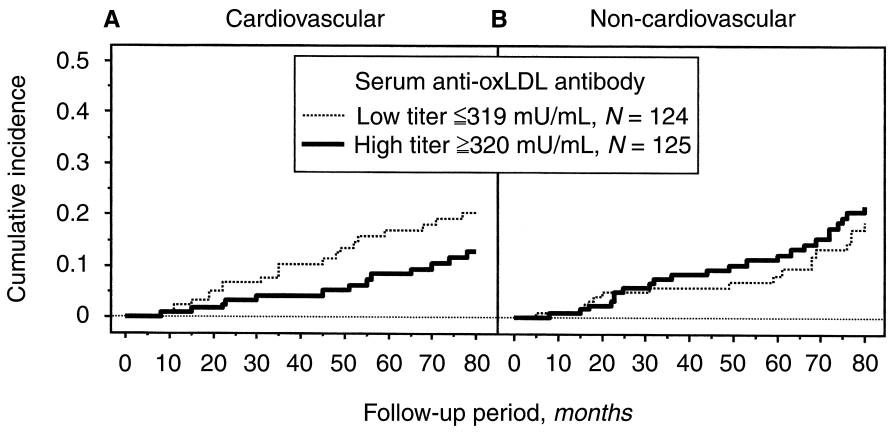

Fig. 2. Anti-oxLDL antibody at baseline and mortality. The cohort of 249 patients with ESRD was divided into two groups according to serum titer of anti-oxLDL antibody titer, and mortality rates were compared by the Kaplan-Meier method followed by log-rank test. Those with a higher titer showed a significantly lower risk of cardiovascular mortality.

Table 2. Univariate association between individual covariates and mortality by the Cox proportional hazards model

	Cardiovascular		Non-cardiovascular	
Variable	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age *per 1 year*	1.08 (1.04–1.12)	<0.0001	1.06 (1.03–1.09)	0.0004
Hemodialysis duration				
long-term vs. short-term	1.99 (0.99–3.98)	0.052	1.41 (0.74–2.66)	0.296
Body mass index *per 1 point*	1.07 (0.95–1.21)	0.271	1.00 (0.89–1.13)	0.944
Serum albumin *per 1 g/L*	0.89 (0.80–1.00)	0.064	0.80 (0.72–0.89)	<0.0001
Hematocrit *per 1%*	1.01 (0.93–1.10)	0.807	1.05 (0.97–1.13)	0.244
C-reactive protein				
elevated vs. normal	3.09 (1.44–6.61)	0.004	2.66 (1.35–5.24)	0.005
Gender				
female vs. male	0.55 (0.28–1.07)	0.079	0.60 (0.32–1.11)	0.106
Diabetes mellitus				
presence vs. absence	2.48 (1.18–5.20)	0.017	3.49 (1.81–6.73)	0.0002
Smoking				
smoker vs. non-smoker	1.02 (0.46–2.26)	0.953	1.14 (0.56–2.34)	0.719
Dyslipidemia				
presence vs. absence	1.42 (0.59–3.44)	0.433	0.71 (0.36–1.39)	0.311
Hypertension				
presence vs. absence	1.76 (0.62–4.98)	0.291	0.94 (0.43–2.04)	0.880
Vascular complication				
presence vs. absence	5.63 (2.87–11.04)	<0.0001	1.74 (0.83–3.66)	0.145
Medication for dyslipidemia				
presence vs. absence	2.91 (0.69–12.19)	0.143	Not calculated[a]	—
Medication for hypertension				
presence vs. absence	1.22 (0.60–2.49)	0.576	0.983 (0.51–1.91)	0.960
Anti-oxLDL antibody				
high vs. low	0.49 (0.24–0.98)	0.045	1.20 (0.64–2.24)	0.573

Duration of hemodialysis was categorized into short-term (<5.4 years) and long-term (5.4 years or longer) according to the median level. C-reactive protein level was categorized into normal (<4.0 mg/dL) and elevated (4.0 mg/dL or higher) based on the normal range. Anti-oxLDL antibody was categorized into two groups by the median value (320 mU/mL). The Table gives hazards ratios (95% confidence intervals) with statistical significance (*P* values).

[a]None of the five patients receiving lipid-lowering drugs (statins) experienced non-cardiovascular death

Factors associated with anti-oxLDL antibody

Factors associated with anti-oxLDL antibody titer were explored. No significant difference was found in anti-oxLDL antibody titer between: genders (338 vs. 299 mU/mL, $P = 0.095$), smokers and non-smokers (322 vs. 288 mU/mL, $P = 0.401$), those with and without diabetes mellitus (314 vs. 364 mU/mL, $P = 0.148$), those with and without hypertension (406 vs. 299 mU/mL, $P = 0.226$), and those with and without vascular complications (319 vs. 321 mU/mL, $P = 0.951$). Also, anti-oxLDL antibody titer did not show a significant correlation with age, duration of hemodialysis treatment, body mass index, serum albumin, serum C-reactive protein levels, or plasma lipid parameters (data not shown).

DISCUSSION

The present study examined whether a serum titer of anti-oxLDL antibody predicts cardiovascular mortality in a cohort of 249 patients with ESRD. To our knowledge, this is the first study to demonstrate that an increased anti-oxLDL antibody titer is an independent

Table 3. Multivariate Cox proportional hazards models of independent predictors of cardiovascular mortality

Covariate	Model 1	Model 2	Model 3	Model 4
Vascular complications	4.04 (2.05–7.96)	4.09 (2.06–8.11)	3.78 (1.91–7.47)	4.01 (2.03–7.92)
presence vs. absence	$P < 0.0001$	$P < 0.0001$	$P = 0.0001$	$P < 0.0001$
Age	1.08 (1.04–1.12)	1.08 (1.03–1.12)	1.07 (1.03–1.11)	1.08 (1.04–1.12)
per 1 year	$P = 0.0002$	$P = 0.0002$	$P = 0.0005$	$P = 0.0002$
Anti-oxLDL antibody	0.46 (0.23–0.95)	0.49 (0.24–1.00)	0.46 (0.23–0.95)	0.45 (0.22–0.93)
high vs. low	$P = 0.037$	$P = 0.05$	$P = 0.037$	$P = 0.031$
C-reactive protein	1.89 (0.97–4.71)	2.00 (0.90–4.42)	2.15 (0.98–4.74)	2.07 (0.34–4.59)
elevated vs. normal	$P = 0.059$	$P = 0.089$	$P = 0.058$	$P = 0.072$
Diabetes mellitus	1.74 (0.92–4.19)	1.83 (0.84–3.97)	1.83 (0.82–4.11)	1.94 (0.90–4.15)
presence vs. absence	$P = 0.083$	$P = 0.128$	$P = 0.143$	$P = 0.089$
Serum albumin	—	0.96 (0.85–1.08)	—	—
per 1 g/L		$P = 0.499$		
Duration of HD	—	—	0.98 (0.91–1.05)	—
per 1 year			$P = 0.522$	
Gender	—	—	—	1.24 (0.62–2.47)
male vs. female				$P = 0.539$
Global model significance	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

The five significant univariate predictors of cardiovascular mortality were forced into the multivariate Cox proportional hazards model to identify independent predictors. The table gives hazards ratios (HR) and 95% confidence intervals (95% CI) with statistical significance (*P* values).

Table 4. Multivariate Cox proportional hazards model of independent predictors of non-cardiovascular mortality

Covariate	HR (95% CI)	*P* value
Serum albumin		
per 1 g/L	0.85 (0.76–0.94)	0.002
Diabetes mellitus		
presence vs. absence	2.63 (1.35–5.12)	0.004
Age *per 1 year*	1.04 (1.01–1.08)	0.011
C-reactive protein		
elevated vs. normal	2.03 (1.02–4.04)	0.044

The four significant univariate predictors of non-cardiovascular mortality were forced into the multivariate Cox proportional hazards model to identify independent predictors. The Table gives hazards ratios (HR) and 95% confidence intervals (95% CI) with statistical significance (*P* values). $P < 0.0001$ for global model significance.

predictor of a lower risk of cardiovascular mortality in patients with ESRD.

There has been a controversy regarding the role of anti-oxLDL antibody in atherosclerotic diseases. In many studies that compared antibody levels between groups with and without advanced atherosclerosis the anti-oxLDL antibody titer was elevated in subjects with advanced atherosclerosis [10–12], whereas other studies failed to confirm such a difference [14–17]. In contrast, still other studies that evaluated the variation of antibody titer within healthy subjects found that anti-oxLDL antibody titer showed an inverse association with intima-media thickness of carotid [19] and femoral [31] arteries in healthy subjects. Importantly, these cross-sectional studies had limitations in showing the causality of the relationship between anti-oxLDL antibody and cardiovascular diseases. In the present prospective cohort study, a higher serum anti-oxLDL antibody titer was independently associated with a lower risk of death from cardiovascular causes, thus showing that anti-oxLDL antibody is an independent predictor of cardiovascular mortality.

It may be confusing that a higher titer of anti-oxLDL antibody predicted a lower risk of cardiovascular death, because the ESRD cohort showed a higher anti-oxLDL antibody titer and a substantially elevated death rate. The raised titer of oxLDL antibody in our ESRD cohort is in agreement with previous studies by Maggi et al [26, 27], although there are other studies that failed to show an increase in antibody titer in smaller numbers of patients with renal failure [32–34]. This apparent discrepancy between the cross-sectional and longitudinal studies can be explained when one considers a change in the cohort members due to death. Namely, in a high risk population like patients with ESRD and those with advanced atherosclerosis, patients with a low titer of anti-oxLDL antibody are more likely to die from cardiovascular causes than those with a high antibody level. Therefore, survivors would show an increased median level of anti-oxLDL antibody that is likely to be detected by cross-sectional studies.

Although we did not address the mechanisms by which anti-oxLDL antibody predicted cardiovascular mortality, we propose several possibilities. First, anti-oxLDL antibody may have anti-atherogenic functions. In animal models, immunization with oxidatively modified LDL remarkably suppressed the development of atherosclerosis [35, 36], whereas immunosuppression with cyclosporine A promoted atherosclerosis [37]. Horrko et al reported that uptake of oxLDL by macrophages via the macrophage scavenger receptor was almost completely inhibited by monoclonal antibodies that recognize oxidized phospholipid epitopes of oxLDL [38]. Therefore, in the presence of anti-oxLDL antibody, oxLDL parti-

cles generated in the subendothelium may back-diffuse into the circulation instead of being taken up by macrophages. In addition, anti-oxLDL antibody may enhance the clearance of oxLDL particles from the circulation. We recently found an inverse correlation between serum anti-oxLDL antibody titer and plasma oxLDL concentration in healthy subjects [18], supporting this possibility. Antibody-bound lipoproteins were shown to be metabolized by macrophages via the Fc-receptor pathway [39]. Immune complex of oxLDL and anti-oxLDL antibody may be taken up by macrophages outside the arterial wall, such as by Kupffer cells in the liver. In fact, intravenously injected ^{125}I-labeled oxLDL was rapidly cleared from the circulation by hepatic Kupffer cells of mice independent of the class A scavenger receptor [40].

Second, although anti-oxLDL antibody itself may not be protective against atherosclerosis, it may have a close association with other pro- or anti-atherogenic factors. The results of our study indicate that the association between anti-oxLDL antibody titer and cardiovascular mortality is independent of pre-existing vascular complications, age, elevated C-reactive protein and presence of diabetes mellitus. Another possibility is that oxLDL may be a causal factor for the higher risk of cardiovascular mortality. In fact, there is a significant inverse association between anti-oxLDL antibody titer and plasma oxLDL concentration in the healthy population, as we reported earlier [18]. Therefore, because plasma oxLDL concentration or immune complexes were not measured, we cannot exclude the possibility that the relationship between anti-oxLDL antibody titer and cardiovascular mortality was confounded by plasma oxLDL concentration that may have a causative role.

Previous studies measured IgG- and IgM-class antibodies against oxLDL. These different classes of antibodies may have a different biological role in atherosclerosis and cardiovascular diseases. Shaw et al showed that naturally occurring IgM antibodies blocked the macrophage uptake of oxLDL in apoE deficient mice [41]. According to Hulthe et al, patients with previous history of myocardial infarction had a lower titer of IgM antibody to oxLDL than those without myocardial infarction [17]. Dotevall et al showed that women with diabetes mellitus, either with or without previous myocardial infarction, had higher IgG titers but lower IgM titers of antibodies against LDL modified with malondialdehide (MDA) [42]. These studies suggest that IgG and IgM antibodies against modified LDL have different roles in atherosclerosis. In patients with renal failure, Maggi reported that significant increase in both IgG and IgM antibodies to either cupper-oxidized LDL or MDA-modified LDL, although they did not report correlation between IgG and IgM titers [27]. Our present study only measured IgG-class anti-oxLDL antibody and found that it predicted cardiovascular mortality in the ESRD cohort. Therefore, the results do not answer the question of the relative importance of IgG and IgM antibodies to oxLDL.

An important issue is what makes the inter-individual variation of anti-oxLDL antibody titer. In our subjects, the anti-oxLDL antibody titer was not associated with age, gender, duration of hemodialysis, smoking, blood pressure, plasma lipids, presence of diabetes mellitus, or presence of vascular complications. The antibody titer may be affected by various genetic and acquired factors influencing antibody production including antigen levels within arterial wall, degree of atherosclerosis, and uremia itself. If so, adequacy of hemodialysis is also a possible factor affecting immune response to oxLDL, although we did not measure the exact dialysis dose, such as Kt/V at baseline.

In summary, the present study has shown that serum titer of anti-oxLDL antibody is an independent predictor of cardiovascular death in a cohort of ESRD patients. Further prospective studies are needed to establish the causality between the antibody titer and outcomes. It is also important to examine different roles of IgG and IgM antibodies in atherosclerosis and cardiovascular diseases.

ACKNOWLEDGMENTS

This work was supported partly by a grant in aid from the Ministry of Education, Culture and Sciences, Japan (No. 07770835), and a grant from Heparin Conference, Tokyo, Japan. The authors gratefully acknowledge the special efforts of Mr. Narutoshi Odaka and Ms. Sugako Muro at Inoue Hospital in accumulating clinical data. The authors also thank the 29 doctors for their kind help in collecting the outcome data.

Reprint requests to Tetsuo Shoji, M.D., Ph.D., Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan.
E-mail: t-shoji@med.osaka-cu.ac.jp

REFERENCES

1. Steinberg D, Parthasarathy S, Carew TE, *et al*: Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 320:915–924, 1989
2. Witztum JL: Immunological response to oxidized LDL. *Atherosclerosis* 131(Suppl):S9–S11, 1997
3. Hansson GK, Holm J, Jonasson L: Detection of activated T lymphocytes in the human atherosclerotic plaque. *Am J Pathol* 135:169–175, 1989
4. Sohma Y, Sasano H, Shiga R, *et al*: Accumulation of plasma cells in atherosclerotic lesions of Watanabe heritable hyperlipidemic rabbits. *Proc Natl Acad Sci USA* 92:4937–4941, 1995
5. Haberland ME, Fong D, Cheng L: Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. *Science* 241:215–218, 1988
6. Palinski W, Rosenfeld ME, Yla-Herttuala S, *et al*: Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci USA* 86:1372–1376, 1989
7. Boyd HC, Gown AM, Wolfbauer G, Chait A: Direct evidence for a protein recognized by a monoclonal antibody against oxidatively modified LDL in atherosclerotic lesions from a Watanabe heritable hyperlipidemic rabbit. *Am J Pathol* 135:815–825, 1989
8. Frostegard J, Wu R, Giscombe R, *et al*: Induction of T-cell activation by oxidized low density lipoprotein. *Arterioscler Thromb* 12:461–467, 1992

9. Yla-Herttuala S, Palinski W, Butler SW, *et al*: Rabbit and human atherosclerotic lesions contain IgG that recognizes epitopes of oxidized LDL. *Arterioscler Thromb* 14:32–40, 1994
10. Maggi E, Chiesa R, Melissano G, *et al*: LDL oxidation in patients with severe carotid atherosclerosis. A study of in vitro and in vivo oxidation markers. *Arterioscler Thromb* 14:1892–1899, 1994
11. Virella G, Virella I, Leman RB, *et al*: Anti-oxidized low-density lipoprotein antibodies in patients with coronary heart disease and normal healthy volunteers. *Int J Clin Lab Res* 23:95–101, 1993
12. Bergmark C, Wu R, de Faire U, *et al*: Patients with early-onset peripheral vascular disease have increased levels of autoantibodies against oxidized LDL. *Arterioscler Thromb Vasc Biol* 15:441–445, 1995
13. Salonen JT, Yla-Herttuala S, Yamamoto R, *et al*: Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet* 339:883–887, 1992
14. Uusitupa MI, Niskanen L, Luoma J, *et al*: Autoantibodies against oxidized LDL do not predict atherosclerotic vascular disease in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 16:1236–1242, 1996
15. Cherubini A, Mecocci P, Senin U, *et al*: Autoantibodies against oxidized low-density lipoproteins in older stroke patients. *J Am Geriatr Soc* 45:125, 1997
16. Schumacher M, Eber B, Tatzber F, *et al*: Transient reduction of autoantibodies against oxidized LDL in patients with acute myocardial infarction. *Free Radic Biol Med* 18:1087–1091, 1995
17. Hulthe J, Wikstrand J, Lidell A, *et al*: Antibody titers against oxidized LDL are not elevated in patients with familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 18:1203–1211, 1998
18. Shoji T, Nishizawa Y, Fukumoto M, *et al*: Inverse relationship between circulating oxidized low density lipoprotein (oxLDL) and anti-oxLDL antibody levels in healthy subjects. *Atherosclerosis* 148:171–177, 2000
19. Fukumoto M, Shoji T, Emoto M, *et al*: Antibodies against oxidized LDL and carotid artery intima-media thickness in a healthy population. *Arterioscler Thromb Vasc Biol* 20:703–707, 2000
20. Kawagishi T, Nishizawa Y, Konishi T, *et al*: High-resolution B-mode ultrasonography in evaluation of atherosclerosis in uremia. *Kidney Int* 48:820–826, 1995
21. Shoji T, Nishizawa Y, Kawagishi T, *et al*: Intermediate-density lipoprotein as an independent risk factor for aortic atherosclerosis in hemodialysis patients. *J Am Soc Nephrol* 9:1277–1284, 1998
22. Shoji T, Kawagishi T, Emoto M, *et al*: Additive impacts of diabetes and renal failure on carotid atherosclerosis. *Atherosclerosis* 153: 257–258, 2000
23. Shoji T, Emoto M, Shinohara K, *et al*: Diabetes mellitus, aortic stiffness, and cardiovascular mortality in end-stage renal disease. *J Am Soc Nephrol* 12:2117–2124, 2001
24. Lindner A, Charra B, Sherrard DJ, Scribner BH: Accelerated atherosclerosis in prolonged maintenance hemodialysis. *N Engl J Med* 290:697–701, 1974
25. Ma KW, Greene EL, Raij L: Cardiovascular risk factors in chronic renal failure and hemodialysis populations. *Am J Kidney Dis* 19: 505–513, 1992
26. Maggi E, Bellazzi R, Falaschi F, *et al*: Enhanced LDL oxidation in uremic patients: An additional mechanism for accelerated atherosclerosis? *Kidney Int* 45:876–883, 1994
27. Maggi E, Bellazzi R, Gazo A, *et al*: Autoantibodies against oxidatively-modified LDL in uremic patients undergoing dialysis. *Kidney Int* 46:869–876, 1994
28. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
29. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 285:2486–2497, 2001
30. The sixth report of the Joint National Committee on prevention: Detection, evaluation, and treatment of high blood pressure. *Arch Intern Med* 157:2413–2446, 1997
31. Hulthe J, Bokemark L, Fagerberg B: Antibodies to oxidized LDL in relation to intima-media thickness in carotid and femoral arteries in 58-year-old subjectively clinically healthy men. *Arterioscler Thromb Vasc Biol* 21:101–107, 2001
32. Bergesio F, Monzani G, Ciuti R, *et al*: Autoantibodies against oxidized LDL in chronic renal failure: Role of renal function, diet, and lipids. *Nephron* 87:127–133, 2001
33. O'Byrne D, Devaraj S, Islam KN, *et al*: Low-density lipoprotein (LDL)-induced monocyte-endothelial cell adhesion, soluble cell adhesion molecules, and autoantibodies to oxidized-LDL in chronic renal failure patients on dialysis therapy. *Metabolism* 50:207–215, 2001
34. Roob JM, Rabold T, Hayn M, *et al*: Ex vivo low-density lipoprotein oxidizability and in vivo lipid peroxidation in patients on CAPD. *Kidney Int* 59(Suppl 78):S128–S136, 2001
35. Palinski W, Miller E, Witztum JL: Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. *Proc Natl Acad Sci USA* 92:821–825, 1995
36. Ameli S, Hultgardh-Nilsson A, Regnstrom J, *et al*: Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol* 16:1074–1079, 1996
37. Roselaar SE, Schonfeld G, Daugherty A: Enhanced development of atherosclerosis in cholesterol-fed rabbits by suppression of cell-mediated immunity. *J Clin Invest* 96:1389–1394, 1995
38. Horkko S, Bird DA, Miller E, *et al*: Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. *J Clin Invest* 103:117–128, 1999
39. Khoo JC, Miller E, Pio F, *et al*: Monoclonal antibodies against LDL further enhance macrophage uptake of LDL aggregates. *Arterioscler Thromb* 12:1258–1266, 1992
40. Ling W, Lougheed M, Suzuki H, *et al*: Oxidized or acetylated low density lipoproteins are rapidly cleared by the liver in mice with disruption of the scavenger receptor class A type I/II gene. *J Clin Invest* 100:244–252, 1997
41. Shaw P, Horkko S, Chang M, *et al*: Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. *J Clin Invest* 105:1731–1740, 2000
42. Dotevall A, Hulthe J, Rosengren A, *et al*: Autoantibodies against oxidized low-density lipoprotein and C-reactive protein are associated with diabetes and myocardial infarction in women. *Clin Sci Lond* 101:523–531, 2001